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Plant Gene Register

Isolation and Characterization of an Oilseed Rape Fructose-1,6-Bisphosphatase cDNA[1]

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A full-length cDNA encoding the cytosolic FBPase has been obtained from 4-d-old *Brassica napus* cv Jet Neuf seedlings (Table I). The cytosolic FBPase (D-Fru-1,6-bisphosphatase 1-phosphohydrolase [EC 3.1.3.11]) catalyzes the formation of Fru 6-P and Pi from Fru-1,6-bisP. This enzyme plays an essential role in plant gluconeogenesis.

The pFBPB clone was obtained by rapid amplification of cDNA ends-PCR (Frohman, 1990). Total RNA was isolated (Laroche and Hopkins, 1987) from cotyledons of 4-d-old seedlings, and mRNA was purified using the PolyATract system (Promega). mRNA was reverse transcribed using SuperScript RNase H^- reverse transcriptase (BRL) according to the manufacturer's instructions using the following oligo(dT)-adapter primer: GAG TCG ACT CTA GAA GTT TTT TTT TTT TTT TTT. cDNA was amplified using forward, GAG AAT TCG A(C/T)G G(A/T)G C(A/C/T)C C(A/T)A T, and reverse, GAG TCG ACT CTA GAA GTT, primers in a reaction buffer consisting of 10 mM Tris-HCl (pH 8.2), 50 mM KCl, 2.0 mM $MgCl_2$, 0.001% gelatin, 1 μM reverse and forward primers, and cDNA corresponding to 5 ng of poly(A^+) RNA in the presence of 0.625 unit of *Taq* polymerase in a total volume of 25 μL. The amplification protocol consisted of 3 cycles at 95°C for 1 min, 39°C for 1 min, and 72°C for 3 min and 35 cycles at 95°C for 1 min, 43°C for 1 min, and 72°C for 3 min. The forward primer was deduced from the NH_2-terminal sequence of a castor bean glyoxysomal alkaline lipase (Maeshima et al., 1987). The amplified product was cloned in T-tailed *Eco*RV Bluescript II KS+ vector (Ausubel et al., 1987). Both strands of the clone were sequenced using Sequenase (Version 2.0, United States Biochemical) for manual sequencing and Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems) for automated sequencing (ABI373A). Interestingly, the amplified product, pFPBB, was only amplified with the reverse primer, since the sequence of the reverse primer, GAG TCG ACT CTA GAA GTT, and its complementary sequence were identified, respectively, at the 5′ and 3′ ends of the cloned fragment as revealed by the sequence data.

Table I. *Characteristics of an FBP cDNA from B. napus*

Organism:
Oilseed rape (*Brassica napus* L.) cv Jet Neuf.
Clone Type; Designation:
cDNA; pFBPB.
Gene Product:
FBPase (cytosolic, EC 3.1.3.11), catalyzes the formation of Fru-6-P and Pi from Fru-1,6-P2.
Source:
RT of mRNA isolated from cotyledons in germinating seeds.
Method of isolation:
Rapid amplification of cDNA ends-PCR.
Method of Identification:
Sequence comparison between GenBank/EMBL data bases.
Features of cDNA:
34-bp untranslated 5′ end; 88-bp untranslated 3′ end; 1020-bp open reading frame.
GC Content:
44% for full-length clone; 46% for open reading frame.
Features of Deduced Amino Acid Sequence:
Open reading frame encodes a polypeptide of 339 amino acids of M_r 37,156. pI = 5.1. Integral protein with transmembrane segment predicted for residues 34–50.
Expression Characteristics and Subcellular Location:
Not determined.
Antibody:
Not available.

The full-length pFBPB clone has 1142 nucleotides, 1020 of which correspond to an open reading frame encoding 339 amino acids. The identity of this clone was deduced from the sequence comparison of nucleotides (77% identical) and amino acids (85% identical, 92% similar) (Altschul et al., 1990) with known sequences of FBPase isolated from spinach (Hur et al., 1992; P14766), potato (X76946), and sugarbeet (M80597).

The deduced composition of the 339 amino acids is very similar to those residues reported for the enzyme cloned from spinach (341), potato (340), and sugarbeet (329). All of the deduced enzymes show a highly conserved (79% identical, 100% similar) transmembrane segment close to the NH_2 extremity (residues 34–50), which classify this protein as integral. The deduced pI of pFBFB of 5.1 is slightly more acidic

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Abbreviation: FBPase, Fru-1,6-bisphosphatase.

than for the corresponding enzyme in the three other species (spinach, 5.35; potato 5.73; and sugarbeet, 5.96). The deduced amino acid sequence exhibits identical residues in 14 of the 15 Fru-6-P-binding sites when compared to the deduced polypeptides from spinach, potato, and sugarbeet; the 19 Fru-2,6-bisP-binding residues or active sites in rapeseed are identical with those from the other plant species (Hur et al., 1992). The two structurally important histidyl residues (255, 313) of FBPase are identical among all species. These specific sites are also conserved in FBPase chloroplast isozymes identified in Arabidopsis (F16P ARA), oilseed rape (BNAFB-PCP), and spinach (F16P SPI). Based on a phylogenic algorithm (Clustal, PC/Gene 6.8), the FBPase sequence from *B. napus* has the lowest level of homology compared to those corresponding cytosolic sequences identified in spinach, sugarbeet, and potato.

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